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A COMPARATIVE STUDY REGARDING THE ASSOCIATION OF ALPHA-2U GLOBULIN WITH THE NEPHROTOXIC MECHANISM OF CERTAIN PETROLEUM-BASED AIR FORCE FUELS

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Adult male rats have a strain, dose, and time-dependent renal proximal tubular degeneration induced by certain hydrocarbon compounds. We are using rat strain variation (Fisher 344 and NCI Black Reiter) and different hydrocarbon compounds (JP-4, JP-8, decalin and trimethylpentane) to investigate the hydrocarbon-induced nephrotoxic response. Histochemical and morphometric evaluation of NCI-Black Reiter rats exposed to JP-8 indicate that this strain undergoes an intermediate form of the hydrocarbon-induced nephrotoxicity when compared to the albino Fisher 344 strain. The intermediate nephrotoxic response of the NCI-Black Reiter rat is characterized by approximately a two-fold increase in the number of acid phosphatase reactive lysosomes in renal tubular cells. The NBR rats did not demonstrate an increase in the size of the individual lysosomes, however, a characteristic lysosomal aggregation pattern occurred in renal tubular cells following hydrocarbon exposure.

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Annual Technical Report (7/1/91-6/30/92)

Prepared by

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August 1992

DISCUSSION AND CONCLUSION

Summary

Adult male rats have a strain, dose, and time-dependent renal proximal tubular degeneration induced by certain hydrocarbon compounds. We are using rat strain variation (Fisher 344 and NCI Black Reiter) and different hydrocarbon compounds (JP-4, JP-8, decalin and trimethylpentane) to investigate the hydrocarbon-induced nephrotoxic response. Histochemical and morphometric evaluation of NCI-Black Reiter rats exposed to JP-8 indicate that this strain undergoes an intermediate form of the hydrocarbon-induced nephrotoxicity when compared to the albino Fisher 344 strain. The intermediate nephrotoxic response of the NCI-Black Reiter rat is characterized by approximately a two-fold increase in the number of acid phosphatase reactive lysosomes in renal tubular cells. The NBR rats did not demonstrate an increase in the size of the individual lysosomes, however, a characteristic lysosomal aggregation pattern occurred in renal tubular cells following hydrocarbon exposure.

Introduction

The exposure of male rats to compounds used in this study results in a nephrotoxic response. The human renal response to hydrocarbon exposure is less clearly defined. Most human studies have focused on leaded gasoline and have failed to demonstrate any consistent association between long term, low level agent exposure and renal disease. However, the potential human health hazard of environmental or occupational hydrocarbon exposure has warranted continued epidemiological and mechanistic studies. Although the primary animal model for human risk assessment of hydrocarbon compounds is the rat, there is considerable controversy regarding the validity of this model. The basis for the controversy is centered on a urinary protein called alpha 2U globulin (A2U) which appears to be unique to the rat. Recent studies have suggested that the NCI-Black Reiter male rat may be less susceptible to the hydrocarbon-induced nephrotoxicity because this rat strain has relatively low levels of blood and urinary A2U.

The principal investigator, in collaboration with toxicologists at AAMRL/THA, Wright-Patterson AFB, is studying the association of A2U with the hydrocarbon-induced nephrotoxic process. The principal objectives of this study are:

1. Use histochemical and morphometric techniques to evaluate the lysosomal alterations in Fisher 344 (A2U +) and NCI Black Reiter (A2U -) rats following exposure to JP-4, JP-8, decalin, and trimethylpentane.
2. Use immunohistochemical (light and electron microscopic) methods to assess the effect of JP-4, JP-8, decalin, and trimethylpentane on the presence of A2U globulin in tubular epithelial cells of the Fisher 344 male rat.

The projected schedule to complete the above objectives is 7/1/90 through 6/30/93. This annual technical report represents our efforts from 7/1/91 through 6/30/92.

Status of the Research

Objective I (Completed)

NCI-Black Reiter (NBR) rats were obtained from the sole source in this country (National Cancer Institute) and a breeding colony was established at AAMRL/VS, Wright-Patterson AFB. NBR rat tissues collected on 8/1/91, 9/13/92, 10/22/92, 12/17/92, and 5/27/92 have been processed by the principal investigator for histochemical and morphometric data. These tissues were recovered following gavage exposures to decalin (12 F344 + 12 NBR), trimethylpentane (12 F344 + 12 NBR), JP-4 (12 F344 + 12 NBR), and JP-8 (12 F344 + 12 NBR). Thirty-two control rats were also processed during the experiment.

Histochemical and morphometric data revealed an a distinct difference in the renal response of F344 and NBR male rats to hydrocarbon exposure. There was approximately a 2-fold increase in the number of lysosomes and lysosomal aggregate formation in renal tubular cells of treated NBR rats. Control animals exposed to saline showed no change in the number or formation of renal tubular lysosomes. Qualitative differences were seen in the degree of renal response with the different hydrocarbon compounds. JP-8 and JP-4 exposures were slightly more disruptive to the histologic architecture of the renal tubular cell than were decalin and trimethylpentane. Representative sections of renal tubular cells stained with the lysosome-specific acid phosphatase stain are shown in Figure 1. Histochemical and morphometric data from Fisher 344 rats revealed the typical enlarged lysosomes with both decalin and JP-8 exposure (Figure 1). We presently believe that the NBR rat demonstrates an intermediate form of hydrocarbon-induced nephrotoxicity. This may be correlated with lower blood and urinary concentrations of A2U. Renal tissues processed using H&E, Mallory Heidenhain's, and Lee's Methylene Blue-Basic Fuchsin stains did not reveal this lysosomal alteration.

Objective 2

The light microscopic immunohistochemistry methods for this project are fully developed, and we presented some of the data at the annual Society of Toxicology meeting (Dallas, TX) and the Midwest Regional Society of Toxicology meeting (Chicago, IL). The presentation was well received and awarded the Victor A. Drill Award for Best Poster Presentation at the Midwest Regional Society of Toxicology meeting. We should complete the light microscopic immunohistochemical analysis of kidney specimens from the exposures listed above (Objective 1) this fall.

Future Plans

We are on schedule for the third year of the proposed 3 year project. We should complete the methods development for electron microscopic immunohistochemistry this fall. A series of F344 and NBR rat exposures to decalin, trimethylpentane, JP-8, and JP-4 are planned in February-March 1993. The electron microscopic immunohistochemical studies should be completed in May 1993.

Publications

Eurell, TE, and Mattie, DR. Lysosomal alterations in F344 and NBR male rats following gavage exposure to decalin and JP-8. *The Toxicologist* 12(1): 428, 1992.

Eurell, TE, and Mattie, DR. The comparison of lysosomal alterations in Fisher 344 and NCI-Black Reiter male rats following gavage exposure to decalin and JP-8. Submitted to *Toxicologic Pathology*

Awards

Davis, MA, and Eurell, TE. Evaluation of the association between alpha 2U globulin and lysosomes in male rats exposed to decalin. This poster received the 1991 Victor A. Drill Award for Best Poster Presentation at the Midwest Regional Meeting of the Society of Toxicology.

Interactions (Coupling Activities)

Dr. Eurell conferred with Dr. David Mattie on 8/1/91, 9/13/92, 10/22/92, 12/17/92, and 5/27/92. During those visits Dr. Eurell presented updates on this project and was involved in tissue collection and processing.

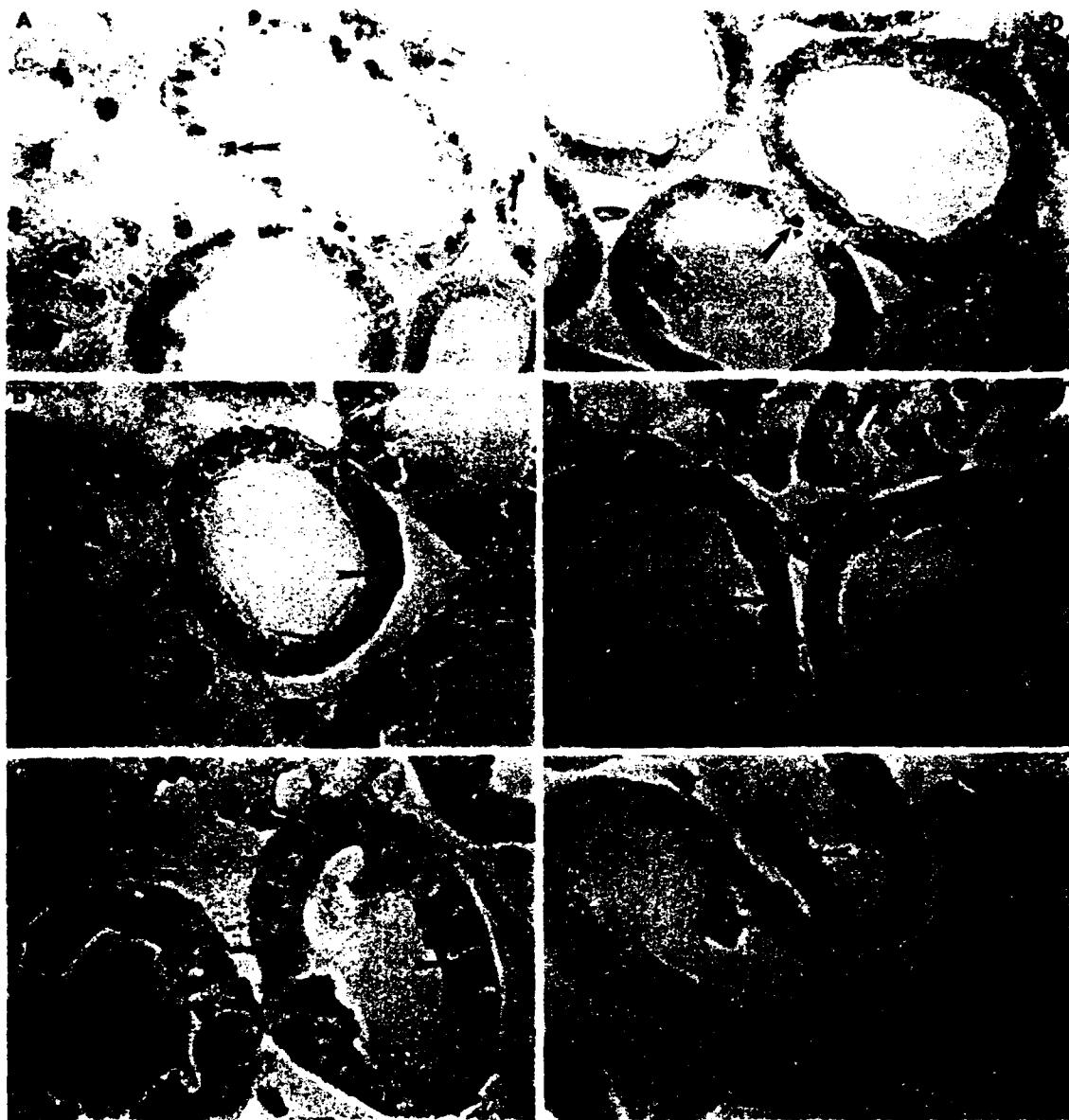


Figure 1. Renal tissue from exposed and control male rats stained with naphthol AS-TR phosphate pararosaniline. Frame A. Control male F344 rat kidney section. The acidophilic droplets (arrow) are due to the acid phosphatase reaction of the lysosomes. Frame B. Kidney section of male F344 male rat exposed to decalin. Note enlarged lysosomes (arrow) distributed along the basal border of the tubular epithelial cell. Frame C. Kidney section of male F344 male rat exposed to JP-8. Note enlarged lysosomes (arrow) distributed along the basal border of the tubular epithelial cell. Frame D. Control male NBR rat kidney section. The acidophilic droplets (arrow) are due to the acid phosphatase reaction of the lysosomes. Frame E. Kidney section of male NBR male rat exposed to decalin. Note the increased number of lysosomes distributed along the median plane of the tubular epithelial cell. Arrow indicates a lysosome cluster. Frame F. Kidney section of male NBR male rat exposed to JP-8. Note the increased number of lysosomes distributed along the median plane of the tubular epithelial cell. Arrow indicates a lysosome cluster. Magnification (1000X).